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## Anticholinergics: Effects on Thermoregulation and Performance in Rats

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MATTHEW, C. B. *Anticholinergics. Effects on thermoregulation and performance in rats.* NEUROSCI BIOBEHAV REV 15(1) 141-146, 1991 - Atropine (AT) induces a dose-dependent increase in rate of rise of core temperature (heating rate) in sedentary heat-stressed rats, a muscarinic anticholinergic (MA) effect which is quantitatively similar to the increase in heating rate seen in heat-exposed men after equivalent atropine dose. In the heat-stressed rat, scopolamine (S) was found to have  $16 \times$  the MA effect of AT and, in the present study, apophen (AP) and trihexyphenidyl (THP) manifested  $0.067 \times$  and  $0.061 \times$  the MA effect of AT. In rats exercising on a treadmill (11 m/min, 6° incline, 26°C), physostigmine (PH) administration resulted in reduced endurance and increased heating rate, both of which were attenuated following AT administration—hypothesized to be a nicotinic anticholinergic (NA) effect. Optimum doses of anticholinergics to reverse the PH-induced decrements were: AT—200  $\mu\text{g/kg}$ , S—8–16  $\mu\text{g/kg}$ , AP—3000  $\mu\text{g/kg}$ , and THP—800  $\mu\text{g/kg}$ . These optimum NA doses for AT, S, and AP were the same as those predicted from their MA potency relative to AT in heat-stressed rats. However, it should be noted that 800  $\mu\text{g/kg}$  of THP is only  $\frac{1}{4}$  of the expected 3200  $\mu\text{g/kg}$  dose of THP based on MA equivalence to AT. Relative MA activities and optimum doses in PH-treated exercising rats appear to be due to differential MA and NA activities. Thus, a combination of both sedentary heat-stressed and exercising rat models may be useful in predicting relative cholinergic effects of new drugs with both MA and NA effects in man.

Anticholinergic	Anticholinesterase	Rat	Model	Nicotinic	Muscarinic	Exercise
Temperature regulation	Hyperthermia					

91-10517

THE effects of acetylcholine can be categorized as muscarinic or nicotinic depending on the type of receptor activated. Stimulation of muscarinic receptors results in generalized vasodilation, decreased heart rate and cardiac contractility, increased secretion of all exocrine glands, including sweat and salivary, increased intestinal and gastric contractions, and increased gastric and tracheo-bronchial secretions (14). Low to moderate doses of an agonist at nicotinic receptors result in stimulation of autonomic ganglia and stimulation at neuromuscular junctions, at high doses receptor blockade and muscle paralysis may result (14). Over stimulation at the neuromuscular junction (nicotinic) results in fasciculations, asynchronous excitation, fatigability, and involuntary twitching (14). The fasciculations and involuntary twitching result in increased metabolic activity and consequent increased heat production.

We have previously reported that atropine (AT, the prototype of muscarinic anticholinergic drugs) induces a dose-response increase in heating rate (rate of rise of core temperature) in the *sedentary heat-stressed rat* (18,25). This is a muscarinic anticholinergic (MA) effect, because it results from inhibition of salivation. In hot environments rats spread saliva over the ventral surface of their bodies for evaporative cooling (16). Both saliva secretion in rodents (18,25) and sweat secretion in humans (6, 20, 21) are inhibited by atropine. Clubleby et al. (5) simultaneously measured sweating and salivation in humans and determined that they were similarly inhibited by atropine. A 2 mg dose of AT will

suppress the sweat rate of humans in the heat by about 40% (7, 8, 30, 31). In the rat, 200  $\mu\text{g/kg}$  is equivalent to 2 mg in man (12), and 200  $\mu\text{g/kg}$  of AT elicits a similar 40% inhibition of water loss via saliva in the rat (18,25). Additionally, the time course of action of AT in the rat closely approximates that seen in humans (25).

Administration of the anticholinesterase physostigmine (PH, 200  $\mu\text{g/kg}$ ) to rats *exercising on a treadmill*, at a dose resulting in an increased heating rate and a decreased endurance (23,26), is accompanied by marked fasciculations and involuntary twitching (nicotinic) (14,26). The decreased endurance could be due to nicotinic-induced over stimulation and fatigability of the running muscles. The increased heating rate of rats exercising after PH administration is related to the muscular activity of running, because PH administration to sedentary rats does not increase core temperature (24, 28, 29). In fact, the tail vasodilation (muscarinic), evident with PH administration, results in decreased not increased heat storage in sedentary rats (26,29). Tail vasodilation dissipates the increased metabolic heat generated in sedentary PH-treated rats but is inadequate for heat dissipation in the exercising rat (26). Further, the increased heating rate in exercising rats appears to be a peripheral effect, since the same increased heating rate results from the administration of pyridostigmine, a quaternary carbamate lacking in central effects at normal dose levels (27). When a rat is running on a treadmill, an increase in salivary water loss (muscarinic) is of no thermoregulatory bene-

TABLE 1  
DRUG EFFECT CHECK LIST FOR RUNNING RATS

Tremors*	none 0	slight 1	moderate 2	violent (convulsing) 3
Salivation†	none 0	licking 1	visible 2	copious 3
Exophthalmos‡	no 0	slight 1	definite 2	
Defecation§	none 0	1-3 pellets 1	>3 pellets 2	diarrhea 3
Run Performance¶	excellent 0	average 1	poor 2	not at all 3
Overall Behavior#	normal 0	slightly over or under 1	moderately over or under 2	convulsing or comatose 3

e.g., Animals given 200 µg/kg of PH alone usually exhibited:

\*Moderate tremors (score of 2).

†Copious dripping saliva (score of 3).

‡Slight to definite exophthalmos (score of 1-2).

§Defecation in excess of 2 pellets (score of 1-3).

¶Poor run performance or inability to run at all (score 2-3).

#Moderately hypoactive (lethargic, score of 2).

fit, because it can not be spread behaviorally. Thus, running rats can dissipate heat only through dilation of tail vessels (29). While the administration of PH should theoretically result in an increased concentration of acetylcholine at all receptors (32), we hypothesize that the decrements in performance of PH-treated exercising rats are due to nicotinic stimulation. If there is improvement in endurance or thermoregulation of PH-treated exercising rats following the administration of anticholinergics, we propose that this is a nicotinic anticholinergic (NA) effect of the drugs.

This study examines the effects of 4 anticholinergic drugs in the sedentary heat-stressed rat and in the exercising PH-treated rat to determine their relative MA and NA potencies. These 4 drugs (atropine, scopolamine, aprophen, and trihexyphenidyl) are being considered, because they have all been proposed as adjuncts to ameliorate side effects induced by PH administration (3, 9, 11, 19, 23, 26).

#### METHOD

##### Animals

Adult male Sprague-Dawley rats (Charles River, CD strain, 510-530 g, N=12/group) were caged individually in wire-bottomed cages in an environmental chamber (4 × 3 × 2 m) at 26°C and 50% rh, and used one time only. Lighting was controlled automatically (on, 0600-1800 h) and Purina rat chow and water were available ad lib except during experimental intervals.

##### Drugs

Aprophen hydrochloride (AP) (Starks Associates, Inc., Buffalo, NY 14213, Lot No. JF14-53-4), atropine sulfate (AT) (Sigma Chemical Company, St. Louis, MO 63178, Lot No. 35F-0648), scopolamine hydrobromide (S) (Boehringer Ingelheim, West Germany, no Lot No.), physostigmine salicylate (PH) (Sandoz, Wander, Roussel Corp. NY, Lot No. 85003.01), and trihexyphenidyl hydrochloride (THP) (Sigma Chemical Co., Lot No. 2F-5098) were dissolved in 0.2 ml of sterile 0.9% saline and administered via lateral tail vein (IV). All anticholinergic drug doses are calculated as the free base, but the physostigmine dose is cal-

culated as µg/kg of the salicylate. To get rat equivalents to human clinical drug dose ranges, the dose/kg in man was multiplied by 7 (12).

##### Sedentary Heat Stress Procedure

To determine the anticholinergic potencies of AP and THP relative to that of AT, a dose [within the equivalent clinical human dose range (14)] of each was administered IV 15 min prior to heat stress. Unrestrained rats were heat stressed in their own cages in a 1 × 2 × 2 m chamber maintained at 41.5°C and 30% rh until a core temperature (T<sub>c</sub>) of 42.6°C was attained. At that time the animals were removed from the heat, weighed, and allowed to cool passively in a 26°C chamber. During heating and cooling, weight loss (as a measure of fluid spread for evaporative cooling), corrected for fecal pellet production, and core temperature (rectal probe inserted 6.5 cm) were monitored. Using the heating rates of these animals, an anticholinergic potency relative to atropine was calculated as previously reported (24).

##### Exercise Procedure

To determine an optimum dose (the dose that comes as close as possible to returning endurance and thermoregulation to control levels) of each anticholinergic drug to be used as an adjunct to PH in the running rat, a range of doses of each of the 4 anticholinergic drugs (AT, AP, S, THP) were evaluated. The dose of each drug calculated to be equivalent to 200 µg/kg (0.69 µM/kg) of AT in the sedentary heat-stress experiments (Table 2) was the initial dose in the exercise studies. Each animal received 2 injections 10 min apart. The first was saline or the appropriate dose of the anticholinergic drug followed by a second injection of saline or 200 µg/kg (0.48 µM/kg) of PH. We have previously reported that administration of this dose and form of PH resulted in 60% inhibition of whole blood cholinesterase (23). Fifteen minutes after drug administration the rats were run (motor driven treadmill, 11 m/min, 6° incline, 26°C, 50% rh, shock avoidance contingency) until exhausted. Exhaustion is defined as the point at which rats do not right themselves when placed on their backs.

During the run and subsequent recovery,  $T_c$  (core temperature) and  $T_t$  (tail skin temperature) were monitored.

The administration of PH alone results in readily detectable side effects. A check list (Table 1) was used to quantitate these side effects and to determine any improvement in these effects resulting from the administration of the 4 adjuncts. The first 4 items were evaluated during the 15 min between the injection of PH and the start of run, and the last 2 were judgments based on performance during the run. All evaluations were made by an observer without knowledge of the treatment group.

#### Statistical Analysis

All values are mean  $\pm$  SE. The data were analyzed by a one-way analysis of variance followed by the Student-Newman-Keuls multiple range test for all pair comparisons. The null hypothesis was rejected at the  $p < 0.05$  level. Values in Figs. 1-4 and Table 3 are given in terms of % of saline controls in order to use only one y-axis for the 3 variables heat rate, run time, and drug effect. Significant differences among all groups were determined on the raw data that these %'s represent.

#### RESULTS

##### Sedentary Heat-Stressed Rats

Rate of rise of core temperature (heating rate) was found to be the most sensitive index of atropine effect in the sedentary heat-stressed rat (26). Therefore, the atropine-induced dose-response increase in heating rate was used to calculate an anticholinergic potency of 16 for S relative to a value of 1 for atropine (Table 2) (24). Table 2 contains the heating rates for the indicated doses of AP and THP determined in this study. Using the method of Matthew et al. (24), these heating rates and doses were used to calculate anticholinergic potency ratios of 0.067 and 0.061 for AP and THP, respectively, relative to a value of 1 for AT. As an alternative means of comparing these 4 drugs, the 200  $\mu\text{g/kg}$  dose of AT was divided by the potency ratio of each drug to determine the dose of each drug that would theoretically inhibit thermoregulation in the heat-stressed rat to the same extent as 200  $\mu\text{g/kg}$  of AT. If there is a dose-response relationship between drug dose and heating rate for S, AP, and THP as has been demonstrated for AT in the sedentary heat-stressed rat (25), the administration of 12  $\mu\text{g/kg}$  of S, 3000  $\mu\text{g/kg}$  of AP, or 3200  $\mu\text{g/kg}$  of THP should elicit a heating rate of 0.087°C/min.

##### Performance Data for Exercising Rats

Figures 1-4 illustrate the results of experiments to determine the dose of each of the 4 anticholinergic drugs tested that optimize PH-decremented endurance and thermoregulation in exercising rats. In each of these figures run time (endurance), heating rate, and drug effect (score on a cholinergic symptom and run performance check list, Table 1) are plotted as a % of saline control values. Separate control and PH alone groups were done for each trial; there were no significant differences among these 4 control groups or among the 4 PH groups. The mean values for run time, heating rate, and drug effect for controls (C) and PH groups across the 4 sets of studies were C—62  $\pm$  6 min, 0.052  $\pm$  0.007 °C/min, and 2.3  $\pm$  0.2; PH—33  $\pm$  4 min, 0.096  $\pm$  0.008°C/min, and 8.9  $\pm$  0.9.

In Fig. 1 note that all of the values are significantly different from controls; therefore, no dose of AT completely restored PH-induced endurance and thermoregulatory decrements. While, all doses of AT significantly improved the drug effect score, only the 200  $\mu\text{g/kg}$  dose of AT significantly improved endurance (run time)

TABLE 2  
MUSCARINIC ANTICHOLINERGIC POTENCY RELATIVE TO ATROPINE

Drug	Dose ( $\mu\text{g/kg}$ ) [ $\mu\text{M/kg}$ ]	Heating Rate (°C/min)	Potency Ratio*	AT=Dose† ( $\mu\text{g/kg}$ )
Saline	—	0.022‡ $\pm 0.001$	—	—
Atropine	200 (0.69)	0.07‡ $\pm 0.004$	1‡	200
Scopolamine	10 (0.03)	0.079‡ $\pm 0.005$	16‡	12
Aprophen	2000 (6.15)	0.072 $\pm 0.004$	0.067	3000
Trihexyphenidyl	400 (1.32)	0.033 $\pm 0.004$	0.061	3200

\*Calculated muscarinic potency relative to atropine (24) corrected for differences in molecular weight.

†Atropine equivalent dose = 200  $\mu\text{g/kg}$  AT/potency ratio. This dose of each drug should elicit the same heating rate in the heat-stressed rat as 200  $\mu\text{g/kg}$  of AT.

‡Values (mean  $\pm$  SE) taken from Matthew et al. (24). The saline group-heating rate was reconfirmed in this experiment.

over that of the PH group. Increasing the AT from 200 to 400  $\mu\text{g/kg}$  increased heating rate and decreased endurance. Therefore, 200  $\mu\text{g/kg}$  AT [equivalent to a 2 mg dose in man (12)] is the optimum dose as an adjunct to PH.

Figure 2 illustrates the results of the S experiment. All of the S-treated groups had drug effect scores that were significantly better than that of the PH group. Although all groups with S exhibited a slightly increased endurance over the group with PH alone, only the 8  $\mu\text{g/kg}$  group manifested a running time that was significantly greater than that of the PH group. Eight and 16  $\mu\text{g/kg}$  S elicited heating rates that were not significantly different from those of the control group. The high heating rate of the 4  $\mu\text{g/kg}$  group and the elevated heating rate of the group receiving 32  $\mu\text{g/kg}$  indicate that these doses are either insufficient or excessive. These results suggest that 8-16  $\mu\text{g/kg}$  of S [equivalent to a

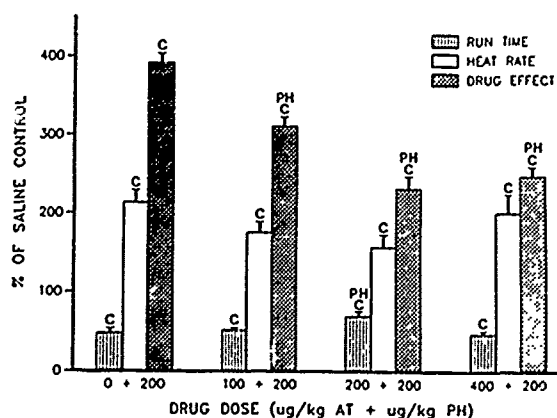


FIG. 1. Run time, heating rate, and drug effect score plotted as a % of saline control values for rats given 200  $\mu\text{g/kg}$  of PH plus the indicated dose of AT prior to exercise. Significant ( $p < 0.05$ ) difference from saline controls is indicated by a C and from the group given PH alone by PH.

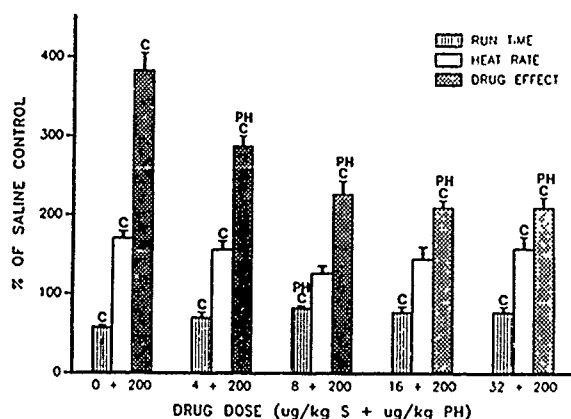


FIG. 2. Run time, heating rate, and drug effect score plotted as a % of saline control values for rats given 200 µg/kg of PH plus the indicated dose of S prior to exercise. Significant ( $p < 0.05$ ) difference from saline controls is indicated by a C and from the group given PH alone by PH.

80–160 µg dose in man (12)] is the optimum dose to be used as an adjunct to PH.

In Fig. 3 it can be observed that all doses of AP significantly improved the drug effects induced by PH administration, and while all doses of AP increased endurance slightly over that of the PH group, none was significantly greater. AP (3 mg/kg) did attenuate the heating rate of PH pretreatment. The group receiving 6 mg/kg of AP had a shorter run time, higher heating rate and greater drug effect score than the group receiving 3 mg/kg, and the 3 mg/kg group had the lowest heating rate and the lowest drug effect score of all the groups receiving both drugs. Therefore, we concluded that 3 mg/kg [equivalent to a 30 mg dose in man (12)] should be the optimum dose when used as an adjunct to PH in the exercising rat.

All three groups receiving both THP and PH (Fig. 4) had drug effect scores that were significantly lower than that of the PH group but also higher than the controls, the 800 µg/kg group had a score closest to that of the control group. Run time was signif-

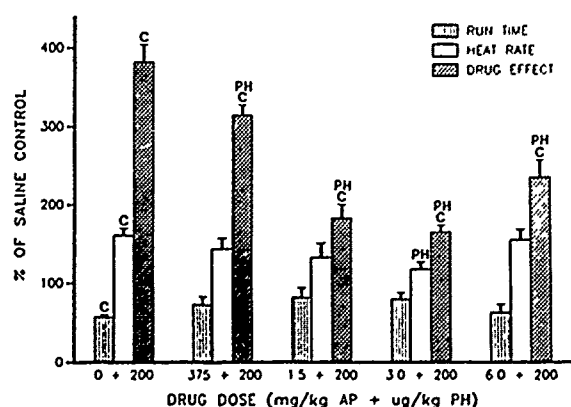


FIG. 3. Run time, heating rate, and drug effect score plotted as a % of saline control values for rats given 200 µg/kg of PH plus the indicated dose of AP prior to exercise. Significant ( $p < 0.05$ ) difference from saline controls is indicated by a C and from the group given PH alone by PH.

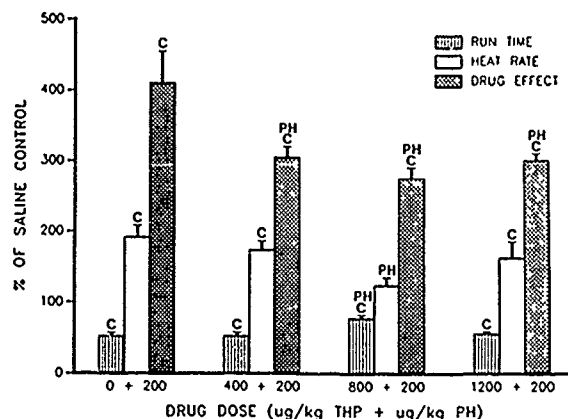


FIG. 4. Run time, heating rate, and drug effect score plotted as a % of saline control values for rats given 200 µg/kg of PH plus the indicated dose of THP prior to exercise. Significant ( $p < 0.05$ ) difference from saline controls is indicated by a C and from the group given PH alone by PH.

icantly lower than control in all 3 groups receiving both drugs, but the group receiving 800 µg/kg THP had a run time that was significantly greater than that of the PH group. The heating rates of the 400 and 1200 µg/kg groups were greater than those of the control group and not different from the PH group, however, the 800 µg/kg group had a heating rate that was significantly lower than that of the PH group and not different from that of controls. From these data it appears that the 800 µg/kg dose of THP [equivalent to 8 mg/70 kg man (12)] is the optimum dose as an adjunct to PH in the running rat.

Table 3 summarizes the results of these 4 experiments. The optimum dose of each drug as an adjunct to PH pretreatment in exercising rats as suggested by our data is noted in the first column. Note that except for THP these are the same values as the MA equivalent doses tabulated in the last column of Table 2. A human dosage, equivalent to the optimum rat dose, was calculated by dividing the rat dose/kg by 7 (12) and then multiplying by 70 kg/adult to get an adult dose. These doses all fall within or below the clinically used range. All of the adjuncts decreased the PH elevated heating rate, but both THP and AP elicited significantly lower heating rates than AT. The run time as a % of control values indicates that the optimum dose of each adjunct increased run time to the same extent. Additionally, the optimum dose of THP in running rats is only 1/4 the AT equivalent dose for THP in the sedentary heat-stressed rat.

#### DISCUSSION

In a running rat model, PH administration results in a dose-dependent decrease in endurance and an increase in rate of rise of core temperature (23). Many of the undesirable effects of PH could be counteracted by simultaneous administration of an anticholinergic (14). This study evaluated the relative abilities of 4 anticholinergic drugs with both central and peripheral sites of action (atropine, scopolamine, apophen and trihexyphenidyl) to ameliorate these PH-induced decrements.

In the present work, 200 µg/kg of PH [previously shown to elicit a 60% inhibition of whole blood cholinesterase (23)] decremented endurance and increased heating rate, and 200 µg/kg of AT did not completely restore endurance to control level. In an other previous study (26), this same dose of PH elicited a 40%

TABLE 3  
COMPARISON OF 4 ANTICHOLINERGIC DRUGS IN  
PH-TREATED RUNNING RATS

Drug*	R Dose† ( $\mu$ g/kg) ( $\mu$ M/kg)	H=Dose‡ (mg/adult)	Clinical§ (mg/adult)	HR % C¶ (%)	RT % C¶ (%)
Atropine	200 (0.69)	2	0.5-10	154	70
Scopolamine	8-16 (0.03-0.05)	0.08-0.16	0.6	140	79
Aprophen	3000 (9.23)	30	—#	115**	80
Trihexyphenidyl	800 (2.64)	8	1-15	111**	77

\*Each drug group received the indicated drug plus 200  $\mu$ g/kg (0.48  $\mu$ M/kg) PH.

†Optimum dose of each drug when used with PH in the running rat.

‡Human (70 kg adult) equivalent dose =  $\frac{1}{4}$  the rat dose on a mg/kg basis (12).

§Range of doses in clinical use (14).

¶Heating rate and run time as % of control values.

#Aprophen is not used clinically in the United States.

\*\*Values are significantly different ( $p < 0.05$ ) from AT group.

inhibition of whole blood cholinesterase and 200  $\mu$ g/kg of AT completely restored endurance and thermoregulation. The source of the PH in the earlier work was Antilirium, an injectable solution, (Forest Pharmaceuticals, Inc., St. Louis, MO), however, in the present study, a fresh solution was made up every day using PH obtained from Sandoz. This difference in source and form of PH could explain the greater levels of inhibition and decremented performance in the present study.

Atropine, a commonly used clinical drug, is the prototype of cholinergic muscarinic blocking agents (14) and, as such, has become a standard for comparison with other anticholinergic drugs. Atropine has also been widely used as a treatment drug for organophosphate (OP) poisoning and is an effective pretreatment against poisoning by OP's when used in combination with PH (10,17). Additionally, AT and PH have each been used clinically to counteract mutually induced side effects (2).

Scopolamine is also primarily a muscarinic anticholinergic drug; apparently, however, it has more potent antimuscarinic properties than atropine (2,19), and S has also been used to antagonize PH effects (19). The central antimuscarinic effects of scopolamine make it valuable as an antinotion sickness drug (14,33). Neither AT nor S have previously been shown to have significant NA effects except at doses far in excess of those used clinically (14).

Aprophen is not a drug in clinical use in the United States, but it is used in other countries as both a pre- and a posttreatment for OP poisoning (15). This drug has antimuscarinic, antinicotinic, anticonvulsant, and local anesthetic properties (22). The in vitro MA properties of AP are  $\frac{1}{4}$  as potent as those of AT (9), and it has been shown to have a greater affinity for nicotinic receptors than AT (1,9).

Alternatively, THP (Artane) has several unique properties unlike the other anticholinergics tested in these experiments. For example, the antisecretory effect of THP on salivary and sweat glands is less than that of AT or S (13,14). This is a potential advantage because THP as an adjunct to PH should not inhibit heat dissipation as much as AT or S (6,24). Although AT and THP both have MA effects, AT blocks both M-1 (central, neuronal) and M-2 (secretory, smooth muscle and cardiac) muscarinic receptors, but THP has a greater specific affinity for the M-1 receptors than for either the secretory or cardiac receptors (4,13). Thus, the tachycardia associated with AT administration might be reduced with THP administration (4).

In the running rat model the optimum doses (Table 3) of AT (200  $\mu$ g/kg), S (8-16  $\mu$ g/kg), and AP (3000  $\mu$ g/kg) for use as adjuncts to PH administration (NA) were precisely the doses equivalent to 200  $\mu$ g/kg of AT in the sedentary heat-stressed rat (Table 2, MA). But, the optimum dose of THP in the running rat (Table 3, 800  $\mu$ g/kg) is only  $\frac{1}{4}$  of the dose equivalent to 200  $\mu$ g/kg of AT in the heat-stressed rat (Table 2, 3200  $\mu$ g/kg). This lower MA effect of THP relative to AT might be expected because of the M-1 specificity of THP. Hence, the optimum NA dose of THP may be far less detrimental to thermoregulation in the heat than the optimum NA doses of AT, S, or AP.

Aprophen has been shown to have greater affinity for nicotinic cholinergic receptors (greater NA effect) than AT (1,9), therefore the demonstrated ability (Table 3) of AP over that of AT to return heating rates of exercising rats closer to control values was expected. THP has been shown to antagonize the effects of PH-induced accumulation of ACh (3,11), but the receptor site involved has not been identified. On the basis of the present work we suggest that if appropriate studies are done, it will be concluded that THP has significant NA effects.

None of the 4 anticholinergic drugs used in this study has been identified as a specific nicotinic antagonist. Therefore, we cannot conclusively say that the PH-induced decrements in exercising rats are nicotinic until another study using a specific nicotinic anticholinergic drug such as d-tubocurarine, mecamylamine or hexamethonium has been done. However, the evidence presented here suggests that the heat-stressed and exercising rat models may be useful in determining relative in vivo MA and NA activities of new drugs.

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